AntiBodyChain
Medical Lab Supplies

PRODUCT INFORMATION

PeliClass sheep anti human IgG2

Kit for quantitative determination of human IgG subclasses in serum and plasma on the Beckman IMMAGE / IMMAGE 800

Article number	**M1892**
Product group	**IgG subclasses IMMAGE**
Techique	**Nephelometry/turbidimetry**

Sanquin · UMC Utrecht · Amsterdam UMC · LUMC Leids Universitair Medisch Centrum · Maastricht UMC+ · Radboudumc university medical center

Instructions for use

Sanquin Reagents B.V.
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

Phone: +31 20 5123599
Fax: +31 20 5123570
reagents@sanquin.nl
www.sanquin.org/reagents

PeliClass human IgG subclass Plus kit	REF **M1895**	IVD CE
PeliClass sheep anti human IgG1 Plus	REF **M1891**	IVD CE
PeliClass sheep anti human IgG2 Plus	REF **M1892**	IVD CE
PeliClass latex sheep anti human IgG3 Plus	REF **M1893**	IVD CE
PeliClass latex sheep anti human IgG4 Plus	REF **M1894**	IVD CE
PeliClass human IgG subclass Plus calibrator set	REF **M1896**	IVD CE
PeliClass human IgG subclass Plus control 1	REF **M1897**	IVD CE
PeliClass human IgG subclass Plus control 2	REF **M1898**	IVD CE

301_v03 06/2018 (en)

For professional use only

– Kit for quantitative determination of human IgG subclasses in serum and plasma on the Beckman IMMAGE® / IMMAGE 800®

CAL	CONTROL	NEPH	NEPH/NIPIA	NIPIA	ORG	≥-≤
Calibrator	Control	Nephelometry	Nephelometry / NIPIA	NIPIA	Origin	Range

General information
Human IgG comprises four subclasses: IgG1, IgG2, IgG3 and IgG4. The biochemical characteristics of the IgG subclasses have been described extensively (1-5). The differences between IgG subclasses are reflected in several biologically important functions such as antigen recognition, complement activation and binding to cell surface receptors. Many studies have revealed that abnormalities in serum levels of IgG subclasses may be associated with various disease states (6). Especially the association of selective IgG2 subclass deficiency with increased susceptibility to viral or bacterial infections has been amply documented (4, 5). Low serum levels of IgG2 or IgG3 have been reported in patients with recurrent upper and lower respiratory tract infections. Others have found an association between very low IgG4 serum concentrations and recurrent sino-pulmonary infections (7). Abnormalities in the serum levels of IgG subclasses have also been observed in autoimmune diseases, neurological disorders and in HIV infections (4, 6).

Principle of the test
The PeliClass™ human IgG subclass **Plus** kit has been designed for fast, reproducible and specific quantification of human IgG subclasses (IgG1, IgG2, IgG3 and IgG4).
The nephelometric determination of IgG1 and IgG2 in serum is based on the specific reaction with a monospecific, highly avid anti-IgG subclass antiserum. The turbidimetric determination of IgG3 and IgG4 in serum is based on the specific reaction with a latex reagent coated with the monospecific, highly avid anti-IgG subclass antibodies for greater analytical sensitivity.
The fractionated sheep anti-human IgG subclass sera are made specific by absorption with isolated gammaglobulins of the unwanted IgG subclasses and, if necessary, by absorption with isolated serum fractions (8-10). The polyclonal reagents in this kit are specific for human IgG subclasses and have been selected for high avidity. The nephelometric and the turbidimetric quantifications are based on the generation of immune complexes, quantified by measuring the scattered light and the light passing through, respectively. The IgG subclass concentrations in the test samples are determined by comparison with a reference curve, obtained with the provided IgG subclass calibrators.
IgG subclass control sera are assayed to check the validity of the reference curves and the accuracy of the IgG subclass determinations.
The IgG subclass levels in the calibrators were determined using the ERM-DA470 as calibrator. The assigned values are 6.210 g/L for IgG1, 3.450 g/L for IgG2, 0.390 g/L for IgG3 and 0.591 g/L for IgG4 and are derived from WHO 67/97 (11).

Storage and stability
All components are stable until the expiry date mentioned on the label when stored at 2-8°C. Transport conditions may differ from storage conditions. Do not freeze the IgG3 and IgG4 latex reagents.
After opening and stored at 2-8°C the components are stable for 1 month, taking the expiry date mentioned on the label into account.
Return the components to 2-8°C after completion of the daily workload.
Replace the evaporation caps with screw caps before storing the reagent cartridges.
The calibration curves are stable for at least 1 month, but control sera should always be used to check the validity of the calibration curves.

Package contents
The PeliClass™ human IgG subclass Plus kit allows quantitative determination of the four human IgG subclasses in 50 tests, including calibrators and controls. All components mentioned on the first page of this package insert can be ordered separately.

Sheep antibodies against human IgG1	1x 2.4 mL	REF M1891
Sheep antibodies against human IgG2	1x 2.9 mL	REF M1892
Latex enhanced sheep antibodies against human IgG3	1x 4.0 mL	REF M1893
Latex enhanced sheep antibodies against human IgG4	1x 4.0 mL	REF M1894
Human IgG subclass calibrator set	7x 1.0 mL	REF M1896
Human IgG subclass control 1	1x 1.0 mL	REF M1897
Human IgG subclass control 2	1x 1.0 mL	REF M1898

The IgG1 and IgG2 antisera are specific liquid sheep sera.
The IgG3 and IgG4 latex reagents are a suspension of polystyrene particles coated with specific sheep antibodies.
The calibrators and controls are liquid human sera. Preservative: NaN_3 0.1% (w/v).

Additional materials and/or equipment
- Beckman Coulter IMMAGE® / IMMAGE 800® Immunochemistry System.
- Beckman Coulter IMMAGE® Buffer1.
- Beckman Coulter IMMAGE® Buffer3.
- Beckman Coulter IMMAGE® Diluent1.
- Beckman Coulter IMMAGE® User Defined Reagent (UDR) cartridges.
- Beckman Coulter Immunoglobuline G reagent (total IgG).

Precautions
The calibrators and controls are liquid human sera. Although the human sera have been tested for the markers of specific disease transmitting agents in accordance with current EU guidelines to GMP and found to be nonreactive, all components of human origin should be considered as potentially infectious. Waste-disposal should be performed according to your laboratory regulations.
The reagent cannot be assumed to be free from infectious agents.
All components are stable until the expiry date mentioned on the label when stored at 2-8°C. Transport conditions may differ from storage conditions. Do not freeze the IgG3 and IgG4 latex reagents.

Test sample handling
Serum and plasma (EDTA and Na-heparin) can be tested. The samples should be as fresh as possible or stored at 2-8°C. If samples are not assayed within 1 week, they should be stored frozen at -18°C to -30°C and can be kept for up to 3 months. Repeated freeze-thaw cycles of the samples may deteriorate the analyte. Lipaemic or turbid samples must be clarified by centrifugation before testing. Samples that cannot be clarified should not be used. The samples should not be manually diluted before use, unless stated otherwise.

Test procedure

Latex reagents require extra washing of cuvets, by selecting "Utilities" from the IMMAGE menu bar, followed by selecting option 7: "Wash Cuvettes". Wash cuvets 1x before testing with latex reagents and wash cuvets 1x after use. In case over 39 latex tests are performed in succession, repeat this procedure.

1 Bring all kit components and test samples to room temperature (18-25°C) and mix gently before use. Avoid bubbles or foam.

2 Prepare the reference curves for the IgG subclasses using the ready-for-use calibrators.

a) **In the IMMAGE® user defined reagents (UDRs) can only select BUF10 and DIL10. IgG1 and IgG2 antisera use BUF1 while IgG3 and IgG4 latex reagents use BUF3. Consequently, these tests should be tested batchwise per buffer. Switch buffer when changing from IgG1 and IgG2 antisera to IgG3 and IgG4 latex reagents and vice versa. All four subclass tests use DIL1 as diluent.**
In the IMMAGE 800® the Beckman buffers can be selected for UDRs.

b) Enter the parameters for the four IgG subclass assay protocols as indicated below (see warning (*) under schemes). In the IMMAGE® BUF10 and DIL10 should be selected from the reagent status options.

c) Enter the IgG subclass concentrations of the ready-for-use calibrators. The IgG subclass concentrations in the various calibrators are summarised in Table 2 of the enclosed information leaflet.

d) Label four UDR cartridges, ensuring that the UDR barcode is not covered. Transfer the antisera and the latex reagents from the vials into compartment A of their designated cartridges and cover with the evaporation caps. Place the cartridges in the reagent compartment.

e) Load the calibrators into sample cups, place them into sample racks and load into the sample carousel. Note: Not all calibrator sera are used for all IgG subclass determinations (see Table 2 of the enclosed information leaflet).
NB: When running the IgG subclass assays together, the calibrator set can be loaded four times into four different sample racks or the calibrations can be performed in succession.

f) Start the measurement of the reference curves. Reference curves are calculated in the calibration approval screen using the third order polynomial fitting.

g) After approval of the reference curves the sample dilution in the assay protocol must be changed to the specified dilution (see schemes).

h) Use the control sera to check the validity of the reference curves. The control sera should be assayed and evaluated in the same way as the test samples. The reference curves are valid as long as the controls are reproduced within their respective ranges stated in Table 3 of the enclosed information leaflet.

3 Load the test samples for the IgG subclass determinations. Assay the samples. The system automatically dilutes test samples according to the programmed dilution. For out of range high (ORHI) results the test should be repeated for IgG2 with a higher sample dilution (1:50) or for IgG1, IgG3 and IgG4 with manual sample predilution. Out of range low (ORLO) results should be remeasured in 1:5 sample dilution for IgG3 and IgG4. Do not measure IgG1 and IgG2 in a lower dilution.

4 The accuracy and precision of the IgG subclass determinations can be checked by repeating the IgG subclass determination in control sera 1 and 2.

5 After use reagents should not be left in the IMMAGE® / IMMAGE 800®. Close the cartridges tightly with the screw cap and store at 2-8°C.

IgG1 IMMAGE parameters

PROTOCOL INFORMATION

Chem Name:	**IgG1**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive Nephelometric**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:			
Buffer:	**BUF10**	Diluent:	**DIL10**
Sample or Dilution Volume:	**10 µL**	Gain:	**3**
Reaction Buffer Volume:	**250 µL**	Cal Dilution	**1:8**
Compartment A Volume:	**40 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**6**	Level 1 = Cal 2	
Replicates:	**1**	Level 2 = Cal 3	
		Level 3 = Cal 4	
		Level 4 = Cal 5	
		Level 5 = Cal 6	
		Level 6 = Cal 7	

IgG2 IMMAGE parameters

PROTOCOL INFORMATION

Chem Name:	**IgG2**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive Nephelometric**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:			
Buffer:	**BUF10**	Diluent:	**DIL10**
Sample or Dilution Volume:	**30 µL**	Gain:	**3**
Reaction Buffer Volume:	**240 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**50 µL**	Sample Dilution:	**1:30***
Compartment B Volume:	**0 µL**	Reaction Time:	**2.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**7**	Level 1 = Cal 1	
Replicates:	**1**	Level 2 = Cal 2	
		Level 3 = Cal 3	
		Level 4 = Cal 4	
		Level 5 = Cal 5	
		Level 6 = Cal 6	
		Level 7 = Cal 7	

IgG3 IMMAGE parameters

PROTOCOL INFORMATION

Chem Name:	**IgG3 Latex**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive NIPIA**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:			
Buffer:	**BUF10**	Diluent:	**DIL10**
Sample or Dilution Volume:	**4 µL**	Gain:	**1**
Reaction Buffer Volume:	**200 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**70 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**6**	Level 1 = Cal 2	
Replicates:	**1**	Level 2 = Cal 3	
		Level 3 = Cal 4	
		Level 4 = Cal 5	
		Level 5 = Cal 6	
		Level 6 = Cal 7	

IgG4 IMMAGE parameters

PROTOCOL INFORMATION

Chem Name:	**IgG4 Latex**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive NIPIA**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:			
Buffer:	**BUF10**	Diluent:	**DIL10**
Sample or Dilution Volume:	**7 µL**	Gain:	**1**
Reaction Buffer Volume:	**195 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**70 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**7**	Level 1 = Cal 1	
Replicates:	**1**	Level 2 = Cal 2	
		Level 3 = Cal 3	
		Level 4 = Cal 4	
		Level 5 = Cal 5	
		Level 6 = Cal 6	
		Level 7 = Cal 7	

IgG1 IMMAGE 800 parameters

PROTOCOL INFORMATION

Chem Name:	**IgG1**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive Nephelometric**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:		Tests per cartridge:	
AGXS Limit:		AGXS Enabled:	□
Buffer:	**BUF1**	Diluent:	**DIL1**
Sample or Dilution Volume:	**10 µL**	Gain:	**3**
Reaction Buffer Volume:	**250 µL**	Cal Dilution	**1:8**
Compartment A Volume:	**40 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**6**	Level 1 = Cal 2	
Replicates:	**1**	Level 2 = Cal 3	
		Level 3 = Cal 4	
Update Level:	**4****	Level 4 = Cal 5	
Replicates:	**1****	Level 5 = Cal 6	
		Level 6 = Cal 7	

IgG2 IMMAGE 800 parameters

PROTOCOL INFORMATION

Chem Name:	**IgG2**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive Nephelometric**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:		Tests per cartridge:	
AGXS Limit:		AGXS Enabled:	□
Buffer:	**BUF1**	Diluent:	**DIL1**
Sample or Dilution Volume:	**30 µL**	Gain:	**3**
Reaction Buffer Volume:	**240 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**50 µL**	Sample Dilution:	**1:30***
Compartment B Volume:	**0 µL**	Reaction Time:	**2.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**7**	Level 1 = Cal 1	
Replicates:	**1**	Level 2 = Cal 2	
		Level 3 = Cal 3	
Update Level:	**4****	Level 4 = Cal 4	
Replicates:	**1****	Level 5 = Cal 5	
		Level 6 = Cal 6	
		Level 7 = Cal 7	

IgG3 IMMAGE 800 parameters

PROTOCOL INFORMATION

Chem Name:	**IgG3 Latex**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive NIPIA**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:		Tests per cartridge:	
AGXS Limit:		AGXS Enabled:	□
Buffer:	**BUF3**	Diluent:	**DIL1**
Sample or Dilution Volume:	**4 µL**	Gain:	**1**
Reaction Buffer Volume:	**200 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**70 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**6**	Level 1 = Cal 2	
Replicates:	**1**	Level 2 = Cal 3	
		Level 3 = Cal 4	
Update Level:	**4****	Level 4 = Cal 5	
Replicates:	**1****	Level 5 = Cal 6	
		Level 6 = Cal 7	

IgG4 IMMAGE 800 parameters

PROTOCOL INFORMATION

Chem Name:	**IgG4 Latex**	Units:	**g/L**
Reagent Lot:		Protocol:	**Non Competitive NIPIA**
Reagent Cartridge Lot:		Reagent Expiration Date:	
Reagent Cartridge S/N:		Tests per cartridge:	
AGXS Limit:		AGXS Enabled:	□
Buffer:	**BUF3**	Diluent:	**DIL1**
Sample or Dilution Volume:	**7 µL**	Gain:	**1**
Reaction Buffer Volume:	**195 µL**	Cal Dilution	**1:5**
Compartment A Volume:	**70 µL**	Sample Dilution:	**1:50***
Compartment B Volume:	**0 µL**	Reaction Time:	**1.5 minutes**

CALIBRATION INFORMATION

		Cal Level	Cal Setpoint
Levels:	**7**	Level 1 = Cal 1	
Replicates:	**1**	Level 2 = Cal 2	
		Level 3 = Cal 3	
Update Level:	**4****	Level 4 = Cal 4	
Replicates:	**1****	Level 5 = Cal 5	
		Level 6 = Cal 6	
		Level 7 = Cal 7	

* When a UDR-cartridge is used for the first time, the “Sample Dilution” field automatically displays the same value as the “Cal Dilution” field. In order to run a sample at a different dilution to that of the calibrator, the chemistry must be first calibrated and approved. Only then can the sample dilution be entered as indicated.

** Since this kit has not been developed for single point calibration, customers should ALWAYS run a full calibration curve.

Interpretation

1. The results are calculated automatically by the system.
2. The IgG subclass concentrations in the control sera should lie within the ranges stated in Table 3 of the enclosed information leaflet. If a result is out of range mentioned in Table 3, remeasure the specific control using a new dilution. If the deviated result is confirmed a new reference curve should be established. In case of invalid control results, patient results should not be released.
3. In order to verify possible antigen excess calculate the sum of the four IgG subclasses and compare this with the total IgG concentration measured with an anti-IgG reagent. The sum of the four IgG subclasses should equal total IgG ± 15%. If the sum of all the subclasses is outside the 15% range of the total IgG value, a rerun should be made using different dilutions.
4. For an evaluation of the IgG subclass concentrations in a test sample, compare the levels found with the normal values for IgG subclasses (see **Reference ranges**).

Assay ranges

Measuring ranges and sensitivity are dependent on the concentration of analytes in the calibrator. Consult Table 2 of the enclosed information leaflet for the kit specific assay ranges.

IgG Subclass	Approximate Initial Measuring range		Approximate Sensitivity	
	Concentration (g/L)	Sample Dilution	Concentration (g/L)	Sample Dilution
IgG1	0.80 – 13.2	1:50	0.80	1:50
IgG2	0.24 – 6.90	1:30	0.24	1:30
IgG3	0.09 – 1.45	1:50	0.009	1:5
IgG4	0.06 – 1.73	1:50	0.006	1:5

Reference ranges

Reference ranges (g/L) for IgG subclasses in serum samples of healthy Caucasian individuals (internal data Sanquin, 10, 12). For other populations separate reference ranges should be obtained.

Age				IgG1	IgG2	IgG3	IgG4
1	-	6	mnth	1.8 - 7.0	0.34 - 2.1	0.14 - 0.80	0.017 - 0.36
6	-	12	mnth	2.0 - 7.7	0.34 - 2.3	0.15 - 0.97	0.012 - 0.43
1	-	1½	yr	2.5 - 8.2	0.38 - 2.4	0.15 -1.07	0.011 - 0.62
1½	-	2	yr	2.9 - 8.5	0.45 - 2.6	0.15 - 1.13	0.011 - 0.79
2	-	3	yr	3.2 - 9.0	0.52 - 2.8	0.14 - 1.20	0.012 - 1.06
3	-	4	yr	3.5 - 9.4	0.63 - 3.0	0.13 - 1.26	0.015 - 1.27
4	-	6	yr	3.7 - 10.0	0.72 - 3.4	0.13 - 1.33	0.017 - 1.58
6	-	9	yr	4.0 - 10.8	0.85 - 4.1	0.13 - 1.42	0.023 - 1.89
9	-	12	yr	4.0 - 11.5	0.98 - 4.8	0.15 - 1.49	0.030 - 2.10
12	-	18	yr	3.7 - 12.8	1.06 - 6.1	0.18 - 1.63	0.035 - 2.30
	>	18	yr	4.9 - 11.4	1.50 - 6.4	0.20 - 1.10	0.080 - 1.40

Specifications

a. Reproducibility

		Within run precision				Between run precision				Total precision			
		serum1	serum 2	serum 3	serum 4	serum1	serum 2	serum 3	serum 4	serum1	serum 2	serum 3	serum 4
IgG1	Conc (g/L)	1.03	3.33	6.14	10.5	1.02	3.43	6.25	10.5	1.04	3.53	6.40	10.7
	cv (%)	2.4	1.5	1.2	1.0	2.8	2.8	3.2	2.4	3.2	3.2	3.3	2.4
	n	10	10	10	10	20	20	20	20	80	80	80	80
	days	1	1	1	1	5	5	5	5	20	20	20	20
	batches									2	2	2	2
IgG2	Conc (g/L)	0.262	1.95	3.48	5.92	0.249	1.96	3.53	5.69	0.244	2.00	3.56	5.73
	cv (%)	2.5	1.1	1.1	5.1	4.6	2.6	3.2	3.9	5.6	3.0	2.9	4.9
	n	10	10	10	10	20	20	20	20	79	80	80	77
	days	1	1	1	1	5	5	5	5	20	20	20	20
	batches									1	1	1	1
IgG3	Conc (g/L)	0.124	0.245	0.456	1.30	0.128	0.241	0.442	1.24	0.130	0.244	0.441	1.24
	cv (%)	4.8	1.5	1.4	1.6	5.0	3.8	3.5	3.9	6.5	5.1	3.7	3.1
	n	10	10	10	10	20	20	20	20	98	98	98	98
	days	1	1	1	1	5	5	5	5	20	20	20	20
	batches									3	3	3	3
IgG4	Conc (g/L)	0.071	0.302	0.539	1.37	0.086	0.315	0.557	1.58	0.080	0.320	0.561	1.47
	cv (%)	6.3	1.4	1.8	4.3	6.5	2.5	2.9	3.9	11.8	4.7	4.6	8.1
	n	10	10	10	10	20	20	20	20	100	100	100	98
	days	1	1	1	1	5	5	5	5	15	15	15	15
	batches									3	3	3	3

CLSI/NCLLS EP5-A2 was used.

b. Comparison of PeliClass™ human IgG subclass kit versus PeliClass™ human IgG subclass Plus kit (x versus y)

The IgG1, IgG2, IgG3 and IgG4 concentrations in sera were determined using this kit and were compared with the corresponding values found with the PeliClass human IgG subclass kit (REF. M1775). The method used for comparison was Passing and Bablok regression. CLSI/NCCLS-EP9-A2 was used. The following correlations were established:

IgG subclass	Slope	Confidence interval	Intercept (g/L)	Confidence interval	n	Correlation
IgG1	0.96	0.96 - 0.96	0.18	0.15 – 0.21	100	1.00
IgG2	0.97	0.96 - 0.99	0.07	0.03 – 0.10	100	0.99
IgG3	0.91	0.86 - 0.96	0.041	0.024 – 0.058	97	0.98
IgG4	0.86	0.83 - 0.88	0.031	0.015 – 0.055	94	0.99
sum	1.01	1.00 - 1.03	0.06	-0.08 – 0.21	95	1.00

c. Batch to batch variations of PeliClass™ human IgG subclass Plus kit

The IgG1, IgG2, IgG3 and IgG4 concentrations in sera were determined using different batches reagents. The method used for comparison was Passing and Bablok regression. CLSI/NCCLS-EP9-A2 was used. The following correlations were established:

IgG subclass	Slope	Confidence interval	Intercept (g/L)	Confidence interval	n	Correlation
IgG1	1.01	0.98 – 1.04	-0.03	-0.25 – 0.20	76	0.99
IgG2	1.01	0.99 – 1.03	-0.05	-0.11 – -0.01	77	1.00
IgG3	1.03	1.01 – 1.04	0.001	-0.003 – 0.007	128	1.00
IgG4	1.02	1.01 – 1.03	-0.009	-0.011 – -0.006	115	1.00

d. Sum subclasses versus total IgG

y = 1.07x - 0.15 (g/L). Where x = total IgG and y = sum subclasses.
Correlation coefficient = 0.991 and n = 38.

e. Interferences

The IgG subclass assays were tested for interferences, using CLSI/NCCLS EP7-A2.
The following substances were tested at the concentrations indicated below and the effects are less than 10%. Two concentrations were tested per IgG subclass. IgG1 2.6 – 6.4 g/L, IgG2 1.6 – 3.6 g/L, IgG3 0.188 – 0.423 g/L and IgG4 0.277 – 0.552 g/L.

Substance	**Test concentration**
Albumin	60 g/L
Bilirubin (conjugated)	20 mg/dL
Bilirubin (unconjugated)	20 mg/dL
Hemoglobin	2 g/L
Reumafactor	50 IU/mL
Triglycerides	37 mmol/L*

*: IgG1 IgG3 and IgG4 assays did not show interferences from triglycerids. For IgG2 only after centrifugation of the sample no interference was detected.

Note:
The values cited for specific performance characteristics of the test represent typical results and are not to be viewed as specifications for this kit.

Limitations

1. User should be trained and familiar with the instrument UDR procedure and test procedure.
2. Unexpected results may be obtained for samples containing substances mentioned in **Specifications e.** at higher levels than tested and for samples containing monoclonal immunoglobulins or circulating immune complexes. These samples should be analysed by another method.
3. Beware of maintenance of the instrument conform the manual. Only use a fully equipped instrument.
4. The finding of a decreased level of one of the IgG subclasses can never provide a definite diagnosis but should rather be considered as an indication of a disturbance of the immune system requiring further diagnostic investigation.
5. While reference curves remain stable for 1 month control sera should always be used to check the validity of the reference curves. When a control is out of range a new reference curve must be made.
6. With a new lot a new reference curve must be made.
7. Do not use any material after the expiry date mentioned on the label.
8. Reagents from different batches are not interchangeable.
9. Any remainder of reagents (e.g. dead volume) should not be mixed with contents of freshly opened vials.
10. Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
11. Always determine total IgG and compare with sum subclasses as a check for possible antigen excess.
12. Do not use the kit components in another instrument than mentioned on the labels.
13. Do not use other system settings than mentioned.

References

1. Shakib F. (Volume editor) Basic and Clinical Aspects of IgG Subclasses (Monographs in Allergy 19) Karger (1986).
2. Shakib F. (Editor) The human IgG subclasses Pergamon Press (1990).
3. Vlug A. et al. Eur. Clin. lab. 8: 26 (1989).
4. Jefferis R. and Kumararatne D.S. Clin. Exp. Immunol. 81: 357 (1990).
5. Hamilton R.C. Clin. Chem. 33: 1707 (1987).
6. Vries E. de et al. Clin. Exp. Immunol. 145: 204 (2006).
7. Beck C.S. and Heiner D.C. Am. Rev. Respir. Dis. 124: 94 (1981).
8. Giessen M. et al. Immunology 27: 655 (1974).
9. Goossen P.C.M. et al. J. Immunol. Methods 40: 339 (1981).
10. Vlug A. et al. Ann. Biol. Clin. 52: 561 (1994).
11. Klein F. et al. Clin. Chem. Acta. 150: 119 (1985).
12. Lepage N. et al. Clin. Biochem. 43: 694 (2010).

Sanquin products are guaranteed to perform as described in the original manufacturer's instructions for use. Strict adherence to the procedures, test layouts and recommended reagents and equipment is essential. Sanquin declines all responsibility arising from any deviation thereof.